Rutin Content of Several Varieties of *Nicotiana rustica* and *N. glauca* [1]

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Introduction

In connection with research being conducted at this Laboratory in an attempt to find materials, other than nicotine, of economic importance in *Nicotiana rustica*, investigation of the rutin content was made. Rutin is obtained commercially from buckwheat (1,2) and is important in medicine for control of capillary fragility (3,4) and certain other hemorrhagic conditions. The rutin contents of several species of *Nicotiana* have been reported (5–9).

The present work was confined to *N. rustica*, varieties Olson 68, Armenia, Prosecknskaia (local), Slepukhinskaia (local), Stalingradskaia and Indian (United Provinces) and to a few analyses of *N. glauca*.

Experimental

The plants were grown on the Laboratory grounds.[3] The whole rustica plant was cut and, within one hour after cutting, the leaf web was stripped from the midrib. The leaf web was then cut into approximately one-inch squares with a knife. A sufficient number of plants was harvested to obtain 1000–1500 g. of this material. It was tossed and tumbled in a large metal container to insure adequate mixing before sampling. Four samples were taken simultaneously, two of approximately175 g. each for duplicate rutin analyses and two of approximaetly 350 g. each for duplicate moisture and nicotine analyses. This technique minimizes sampling error (10) as concordant analytical results on duplicate samples could be realized in this manner. This was not true of any other sampling techniques tried.

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[1] Report of a study made under the Research and Marketing Act of 1946.

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[3] Olson 68 is a standard American variety. The four Russian varieties were obtanied through the Russian Embassy; the Indian variety was obtained from the Indian Department of Agriculture.

The plants were analyzed at frequent intervals throughout the growing season. All analyses were made on fresh green leaves of the healthy plant, unless otherwise noted. The method for determining rutin was based on work by Naghski, *et al.* (11), of this Laboratory. The fresh material was extracted with acetone, and the resulting extracts were concentrated until free of acetone. The residue was boiled with sufficient water to dissolve the rutin, and the water-insoluble fats and pigments were removed by filtration. The insoluble residue and filter paper were again extracted with a small quantity of boiling water and filtered. The combined filtrates were stored at room temperature overnight and then in a refrigerator until precipitation of the rutin was complete (from 1 to 7 days, depending on the quantity). The precipitate was recovered on a tared Gooch crucible, dried at 110°, and calculated as percent crude rutin. Rutin prepared in this manner was approximately 85% pure (11). Moisture was determined by drying at 110°. Most of the nicotine analyses were made by an ultraviolet absorption method (12), but some were made by silicotungstic acid precipitation (13). Results obtained by the two methods agreed.

Results and Discussion

The results of the analyses are summarized in Table I. Table II gives the maximum weights of leaves, of nicotine, and of rutin per plant.

For each variety, there is evidence of two maxima in respect to rutin content. These maxima occurred 11 to 30 days apart, and since the varieties were planted within 3 days of each other, the maxima would seem to result from the natural physiological functions of the plant. Although there was a dry period in the growing season necessitating irrigation, the minima did not correspond to this period.

From previous investigation on *N. rustica*, it was known that nicotine content per plant increases steadily to maturity. Fig. 1 shows how rutin, nicotine, and plant weight vary with the age of the plant, using the data of Olson 68 as an example. Although absolute values for the other varieties studied are different, the trends shown here are representative of all. In general, the date at which the maximum nicotine content of a given variety was reached, coincided with the date of maximum weight of leaves per plant to 6 days thereafter. Maximum rutin occurred between the leaf weight maximum and nicotine maximum. Since this rutin maximum was sharp, the selection of the harvest date would be critical, if it is desired to obtain both rutin and nicotine from the plant.

Olson 68 was superior to all other rustica varieties studied in respect to rutin, nicotine, and leaf weight per plant. *Nicotiana glauca*, however, was far superior to rustica in rutin content. Samples of *N. glauca* analyzed 75, 87, and 94 days after transplanting contained 1.18, 1.34,

and 1.88% rutin, respectively, on a moisture-free basis. A sample of suckers (lateral shoots) taken 94 days after transplanting contained 2.08% rutin. The *N. glauca* was sampled by stripping about one-third of the mature lower leaves from each plant each time analyses were

TABLE I

Weight of Leaves and Rutin and Nicotine Content of Nicotiana rustica *at Various Stages of Growth*

Days from transplanting	Weight of leaves per plant[a,b]	Rutin content[a]	Nicotine content[a]	Weight of rutin per plant[b]	Weight of nicotine per plant[b]
			Olson 68		
	g.	%	%	*g.*	*g.*
5	0.3	0.47	—	<0.01	—
26	5.1	0.51	1.05	0.03	0.05
39	10.6	0.66	1.49	0.07	0.16
49	27.7	0.59	2.00	0.16	0.55
63	55.1	0.52	3.16	0.29	1.74
77	70.8	0.42	5.98	0.30	4.23
85	105.2	0.22	5.97	0.23	6.28
91	106.3	0.43	7.44	0.46	7.90
98	84.2	0.23	9.48	0.19	7.98
105	71.2	trace	8.93	trace	6.36
			Armenia		
40	20.8	0.82	1.75	0.17	0.36
66	50.6	0.89	5.29	0.45	2.68
73	64.5	0.56	5.81	0.36	3.75
80	65.9	0.47	7.04	0.31	4.64
88	67.3	0.58	6.58	0.39	4.43
94	58.0	0.54	5.66	0.31	3.28
			Prosecknskaia (local)		
38	20.1	0.73	2.35	0.15	0.47
66	59.7	0.55	5.70	0.33	3.40
73	78.9	0.37	5.63	0.30	4.23
80	65.9	0.37	5.57	0.30	4.57
88	64.0	0.31	5.88	0.20	3.76
94	63.4	none	5.97	none	3.78

TABLE I *Continued*

Days from transplanting	Weight of leaves per plant[a] [b]	Rutin content[a]	Nicotine content[a]	Weight of rutin per plant[b]	Weight of nicotine per plant[b]
			Slepukhinskaia (local)		
	g.	%	%	*g.*	*g.*
38	25.5	0.70	2.27	0.18	0.58
68	62.5	0.39	5.47	0.25	3.42
74	72.9	0.33	5.61	0.24	4.09
82	80.6	0.44	4.28	0.36	3.45
88	72.8	0.31	5.45	0.23	3.97
94	74.5	trace	5.25	trace	3.91
			Stalingradskaia		
38	20.6	0.72	1.97	0.15	0.40
68	52.4	0.37	5.55	0.19	2.91
74	68.0	0.31	4.98	0.21	3.38
82	73.4	0.36	4.67	0.27	3.43
89	72.0	0.40	4.90	0.29	3.53
94	68.2	0.12	4.64	0.08	3.16
			Indian (United Provinces)		
51	37.7	0.46	0.76	0.17	0.29
65	43.6	0.58	0.34	0.25	1.02
71	58.7	0.31	3.12	0.18	1.84
79	67.7	0.44	5.05	0.30	3.42
86	63.5	0.44	6.52	0.28	4.14
93	45.9	0.31	5.97	0.14	2.74

[a] Moisture-free basis.

[b] "Per plant" refers to leaf web only exclusive of mid-rib, petiole, or stalk of the plant. Although there may be nicotine and rutin in these other portions of the plant, analyses were not made on them.

made. This did not permit the weight per plant to be determined for this species. Suckers and flower heads of the rustica varieties, examined separately, contained an average of 0.4% rutin 38 to 42 days after transplanting. A sample of frenched, virus-infected leaves of Olson 68 taken at 86 days contained 0.45% rutin, which is about double the amount found in the normal plant at this age. However, since the diseased plants were about one-half the size of the normal plant, the quantity of rutin per plant was about the same as that of the normal.

TABLE II

Age at which Maximum Weights of Leaves, Rutin, and Nicotine were Attained in Six Varieties of Nicotiana rustica

Variety	Max. weight of leaves per plant		Max. weight of nicotine per plant		First max. weight of rutin per plant		Second max. weight of rutin per plant	
	g.	*age, days*	*g.*	*age, days*	*g.*	*age, days*	*g.*	*age, days*
Olson 68	108	88	8.2	94	0.31	72	0.46	91
Armenia	67	85	4.8	83	0.46	67	0.39	88
Prosecknskaia	82	80	4.6	79	0.33	65	0.30	80
Slepukhinskaia	81	83	4.2	78	0.26	55	0.36	82
Stalingradskaia	73	82	3.5	85	0.18	58	0.30	88
Indian (United Provinces)	68	80	4.2	85	0.25	65	0.31	81

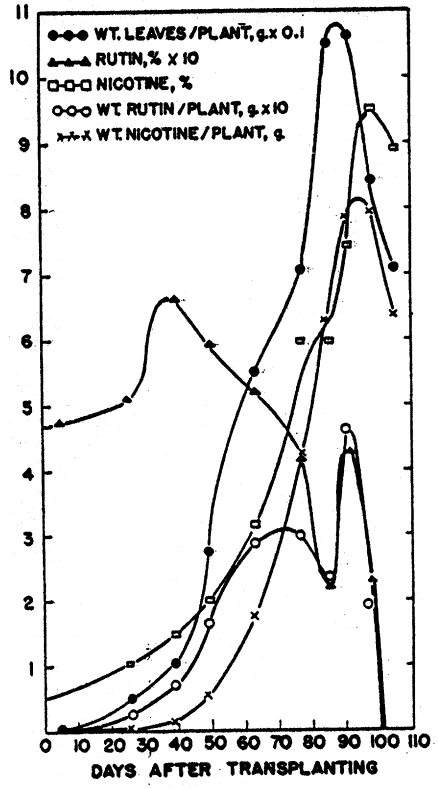

FIG. 1. Weight of leaves and rutin and nicotine contents of *Nicotiana rustica*, variety Olson 68, at various stages of growth. (Moisture-free basis.)

Acknowledgment

The authors express their thanks to C. O. Willits, J. Naghski and C. Ricciuti for helpful suggestions and for the moisture analyses.

Summary

Rutin was found in six varieties of *Nicotiana rustica* not previously reported. The nicotine content attained a single maximum percentage within 6 days of maxiumm leaf weight. Although there is some evidence that the rutin content characteristically showed two peaks, from 11 to 30 days apart, the second and highest occurring between leaf weight and nicotine maxima, the quantity of samples employed might not lead to a valid conclusion. The variety *Prosecknskaia*, for instance, did not show two rutin peaks. Larger amounts of rutin (up to 2%) were found in *Nicotiana glauca* than have been reported previously.

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